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Invasion success of *Fibrillanosema crangonycis*, n.sp., n.g.: a novel vertically transmitted microsporidian parasite from the invasive amphipod host *Crangonyx pseudogracilis*

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Abstract

Parasitism is known to be an important factor in determining the success of biological invasions. Here we examine *Crangonyx pseudogracilis*, a North American amphipod invasive in the United Kingdom and describe a novel microsporidium, *Fibrillanosema crangonycis* n.sp., n.g. The primary site of infection is the female gonad and the parasite is transovarially transmitted to the eggs. PCR screening reveals a female bias in the distribution of parasites (96.6% of females, $N = 29$; 22.2% of males, $N = 27$), which is indicative of host sex ratio distortion. The morphological and molecular characterisations of this new microsporidium place it outside all currently established genera. On the basis of these differences, we erect the new genus *Fibrillanosema* n.g. While *F. crangonycis* is morphologically identical to uncharacterised microsporidia from populations of North American amphipods, it is distinct from microsporidia found in European populations of amphipods. These data support the hypothesis that vertically transmitted parasites may be selectively retained during invasion events. Furthermore where vertical transmission is combined with host sex ratio distortion these parasites may directly enhance host invasion success through increased rates of population growth.

Keywords: Amphipoda; Microsporidia; Molecular phylogeny; Invasion; Vertical transmission; *Crangonyx pseudogracilis*

1. Introduction

Parasites can play an important role in determining the outcome of biological invasions and in structuring communities (Settle and Wilson, 1990; Bauer et al., 2000; Rushton et al., 2000; MacNeil et al., 2003a). These manipulations can ensue from parasite induced modulation of host behaviour and within habitat distribution (Bauer et al., 2000; MacNeil et al., 2003b) or via mediation of competitive and predatory interactions through differential disease susceptibility (Settle and Wilson, 1990; Rushton et al., 2000; MacNeil et al., 2003a). Introduced species are often affected by fewer parasite species and, when infected, suffer lower parasite prevalences than do native populations (Dunn and Dick, 1998; Torchin et al., 2002, 2003). The success of invading species may result from their escape from natural enemies during the process of invasion (Shea and Chesson, 2002; Mitchel and Power, 2003; Wolf, 2003).

Exotic amphipod crustaceans have invaded many native communities throughout the world (Conlan, 1994). In Ireland, the invasion success of *Gammarus pulex* and *Gammarus tigrinus* is dependent on parasitism of native *Gammarus duebeni celticus* by a microsporidian parasite *Pleistophora mulleri* (MacNeil et al., 2003a; Terry et al., 2003) which alters predation hierarchies (MacNeil et al., 2003a). In addition to *P. muelleri*, native UK amphipods are host to the microsporidia *Nosema granulosis* (Terry et al., 1999) and *Microsporidium sp.* (Hogg et al., 2002; Ironside et al., 2003), which cause feminisation of the host (Table 1). The North American amphipod *Crangonyx pseudogracilis* has successfully invaded UK communities of native freshwater gammarids (Conlan, 1994; MacNeil et al.,

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Table 1
Microsporidia present in North American and European amphipods

Host	Microsporidian	Location	A	B	C	D	E	F	G	H
Crangonyx pseudogracilis	*Nosema* sp.	US	1	–	O	11.0 × 3.4	–	–	–	–
	Fibrillanosema crangonycis	UK		G	E	5.6 × 2.1	A	I; 19–26; 1	D	(D)
	Fig. 14	US		H	O	3.7 × 1.6	A	I; 10; 1	D	–
Crangonyx floridanus	Fig. 15	US		G	E	7.1 × 2.7	A	I; 24; 1	D	–
Crangonyx richmondensis	*Amblyospora amphipodae*	US	2	H	B	4.9 × 3.2	S	A; 9; 2	D	O
				H	C	9.7 × 3.7	A	A; 40; 2 +	D	–
Crangonyx serratus	*Thelohania* sp.	US	1	–	–	6.0 × 3.0	–	–	–	–
Gammarus duebeni	*Gurleya polonica*	Poland	3	M	E	4.8 × 2.4	S	I; 20; 2	M	T
	Microsporidium sp. A	UK	4, 11							
	Nosema granulosis	UK	5	G	O	3.8 × 1.2	A	I; 3; 1	D	D
	Octosporea effeminans	Germany	6	G	C	5.0 × 1.5	–	–	B	O
	Thelohania hereditaria	Germany	8	MGA	P	4.0 × 1.75	S	–	B	O
Gammarus pulex	*Nosema rivulogammari*	Sweden	9	M	O	1.75 × 1.2	A	I; 8; 1	B	D/T
				M	P	4.4 × 1.8	A	A; 14; 1	B	D/T
	Pleistophora mulleri	Belgium	7, 10	M	P	4.0 × 2.1	S	A; 15; 2	M	P
	Thelohania muelleri	Sweden	9	MA	P	3.2 × 1.8	S	I; 7; 1	B	O

(A) 1, Johnson and Brooks (1968); 2, Hazard and Oldacre (1976); 3, Wita et al. (1999); 4, Ironside et al. (2003); 5, Terry et al. (1999); 6, Bulnheim and Vavra (1968); 7, Terry et al. (2003); 8, Bulnheim (1971); 9, Larsson (1983); 10, Van Ryckeghem (1930); 11, Terry et al. (In prep.) (B) Site of Infection: *H*epatopancreas, *M*usculature, *G*onads, *A*dipose. (C) Spore shape: *E*longated oval, *O*val, *B*roadly oval, *C*ylindrical, *P*yriform. (D) Approximate spore size (comparison dubious as some are from fresh and some from fixed spores; mean has been calculated when range given). (E) Envelope: *A*bsent; *S*porophorous vesicle. (F) Polar Filament: *I*sofilar, *A*nisofilar; number of turns; number of rows. (G) Nuclei: *D*iplokaryon, *M*onokaryon, *B*oth. (H) Sporulation sequence: *D*isporoblastic, *T*etrasporoblastic, *O*ctosporoblastic, *P*olysporous.

2003a) since it was first recorded in the 1930s (Gledhill et al., 1993). The invasion success of *C. pseudogracilis* has been attributed to its toleration of a broad range of salinity, temperature, pollution and dissolved oxygen regimes, maintaining a high reproductive output even under adverse environmental conditions (Dick et al., 1998). Several microsporidia have been reported from North American populations of *Crangonyx* sp. These include *Amblyospora amphipodae* (Hazard and Oldacre, 1976) and species attributed to the genera *Thelohania* and *Nosema* (Johnson and Brooks, 1968). However, there has been no attempt to establish whether these parasites have successfully invaded with their host. Here we screen a UK population of invasive *C. pseudogracilis* to test for the presence of microsporidia and to consider their impact on invasion success.

2. Materials and methods

The invasive amphipod *C. pseudogracilis* was collected from a pond in Middleton Park, Leeds, UK (1° 32.5′ W 53° 45.1′N). Animals were collected by a standard kick sampling regime (MacNeil et al., 2001). To measure parasite prevalence we screened 56 adult male and female animals, selected at random from the sample population, for microsporidian infection using universal PCR primers. We would predict that a parasite that either feminises or kills male offspring will be found in females more often than in males. We therefore compared the prevalence of the parasite in females and males to provide evidence of host sex ratio distortion. To test for sex ratio distortion gonadal tissue from 27 males and 29 females was screened. In addition, broods of embryos from seven gravid females were also screened to look for evidence of vertical transmission. The parasite was characterised via ultrastructural examination and molecular phylogenetic analysis of partial small subunit (SSU), internal transcribed spacer (ITS) and partial large subunit (LSU) ribosomal DNA (rDNA) gene sequence.

Eight infected adult hosts were prepared for electron microscopy. Animals were halved; one-half was processed for PCR identification of the parasite and the other half was fixed and embedded in Spurr resin for light microscopy (LM) and electron microscopy (EM) observation (Terry et al., 2003). Additionally, we examined archived EM images of microsporidia infected *Crangonyx* spp. that had been collected from Florida, (1982) Louisiana (1984), and North Carolina (1982) USA. Electron microscopy material from the USA was prepared according to the methods in Becnel (1997).

We used a PCR screen to measure parasite prevalence in the population and to look for evidence of vertical transmission. We dissected 27 males and 29 females, discarding the head and gut, and screened the reproductive and non-reproductive tissues for microsporidia. To screen for microsporidian infection and generate rDNA sequence, DNA from *C. pseudogracilis* was extracted and purified following methods of Terry et al., (2003). PCR was carried out using microsporidian rDNA specific primers, 530SSUf

and 580LSUr (Vossbrinck et al., 1993) and the internal primers hg4f and hg4r (Gatehouse and Malone, 1998). Amplifications were performed in a robocycler 96 gradient PCR machine (Stratagene Europe) in 25 µl volumes containing 10 pmol of each primer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$ and 0.625 Units of thermoprime Taq polymerase in Reddymix buffer (Abgene, Surrey, UK) (Terry et al., 2003). The amplification program consisted of 95 °C for 5 min, followed by 40 cycles of 95 °C for 50 s, 50 °C for 70 s and 72 °C for 90 s and completed with a final elongation step performed at 72 °C for 10 min. PCR products were electrophoresed through 1.5% agarose gels and visualised by ethidium bromide staining.

QIAquick gel extraction kits (Qiagen Inc, Sussex, UK) and Wizard PCR Preps (Promega, Southampton, UK) were used to clean up the PCR products, which were then sequenced at the Natural History Museum, London. The partial SSUrDNA, ITS and partial LSUrDNA sequence has been deposited in GenBank, Accession Number (AY364098 (applied for 8 August 2003)). Using the FASTA program (European Bioinformatics Institute), a homology search was performed. The rDNA sequences of 22 microsporidia were obtained from GenBank along with the fungus *Basidiobolus ranarum* (Fungus: Zygomycetes; accession number D29946) which was selected as the outgroup species (Table 2). Bioedit (Hall, 2001) was used to align the sequence with other microsporidian rDNA sequences. The phylogenetic analysis was performed only on those portions of the sequences that could be unambiguously aligned. Parsimony, neighbour joining, and maximum likelihood analyses were conducted using PAUP*[b10] (Swofford, 2002) and a modification of the GTR + I + G substitution model generated by deleting factors from the model that caused a reduction of the log likelihood value (ln L) of no more than one. Nodal support was assessed by bootstrap analysis (100 replicates).

Table 2
GenBank rDNA accession numbers and host information for microsporidia used in the phylogenetic analysis

Organism	Host	Accession number
Amblyospora californica	*Culex tarsalis*	U68473
Antonospora scoticae	*Andrena scotica*	AF024655
Bacillidium sp.	*Lumbriculus* sp.	AF104087
Brachiola algerae	Anopheline mosquitoes	AF069063
Bryonosema plumatella	*Plumatella mittens*	AF484692
Culicospora lunata	*Culex pilosus*	AF027683
Edhazardia aedis	*Aedis aegypti*	AF027684
Encephalitozoon cuniculi	*Homo sapiens*	L39107
Encephalitozoon hellem	*Homo sapiens*	L39108
Enterocytozoon bieneusi	*Homo sapiens*	AF024657
Glugea atherinae	*Atherina presbyter*	U15987
Janacekia debaisieuxi	*Simulium* sp.	AJ252950
Nosema bombycis	*Bombyx mori*	L39111
Nosema granulosis	*Gammarus duebeni*	AJ011833
Nucleospora salmonis	Salmonid fish	U78176
Pseudonosema cristatellae	*Cristatella mucedo*	AF484694
Spraguea lophii	*Lophius americanus*	AF033197
Tracipleistophora hominis	*Homo sapiens*	AJ002605
Thelohania solenopsae	*Solenopsis invicta*	AF134305
Trichonosema pectinatellae	*Pectinatella magnifica*	AF484695
Vairimorpha imperfecta	*Plutella xylostella*	AJ131645
Visvesvaria acridophagus	Mosquitoes	AF024658

3. Results

Examination of *C. pseudogracilis* from Middleton Park, Leeds, UK revealed infection with a microsporidian parasite that was primarily evident in the gonads of female hosts. Field collected females often harboured a discrete white mass under the cuticle (Fig. 1) and dissection revealed that these white masses were ovaries that were heavily infected with microsporidian spores to the extent that the ovaries were little more than a sac of parasites. These masses were never observed in males. The PCR screen revealed an overall parasite prevalence of 60.1% (34/56). A *G*-test was applied under the null hypothesis that the frequency of parasites would be the same in males and females. The parasite was found to have a sex-biased distribution with significant overrepresentation in female hosts (96.6%; 28/29) compared to male hosts (22.2%; 6/27; $G_{adj} = 33.58$, 1 d.f., $P < 0.01$; Table 3). Furthermore, the parasite was detected in the broods of six out of seven infected females (85.7%), which suggests that the parasite is vertically transmitted.

Table 3
Fibrillanosema crangonycis prevalence in *Crangonyx pseudogracilis*, (percent infected) in gonad and total tissue

Females $N = 29$		Males $N = 27$		Broods $N = 7$
Ovaries	Total	Testes	Total	Total
27 (93.1)	28 (96.6)	4 (14.8)	6 (22.2)	6 (85.7)

Examination of semi-thin sections from adult female hosts with light microscopy revealed that the infection was contained within the ovaries and caused severe pathogenesis (Fig. 2). Infection was often bilaterally asymmetrical with one ovary disrupted by spores and the other harbouring a light infection (Fig. 2). In heavily infected ovaries little or none of the original structure of the ovary remained and spore masses had spread to the tissue surrounding the gonad (Fig. 2). Ultrastructural observation revealed that infected cells lacked internal organisation and cell organelles were degenerate (Fig. 3). All stages of the parasite life cycle were seen in host follicle cells (Fig. 4) and in oocytes (Fig. 5). Meronts, sporonts, and sporoblasts were never seen in groupings or within an interfacial envelope. Spores were seen free in host cells with the exception of the occasional grouping (Fig. 6) of a maximum of eight spores in a membrane ($N = 5$). It was unclear whether this membrane

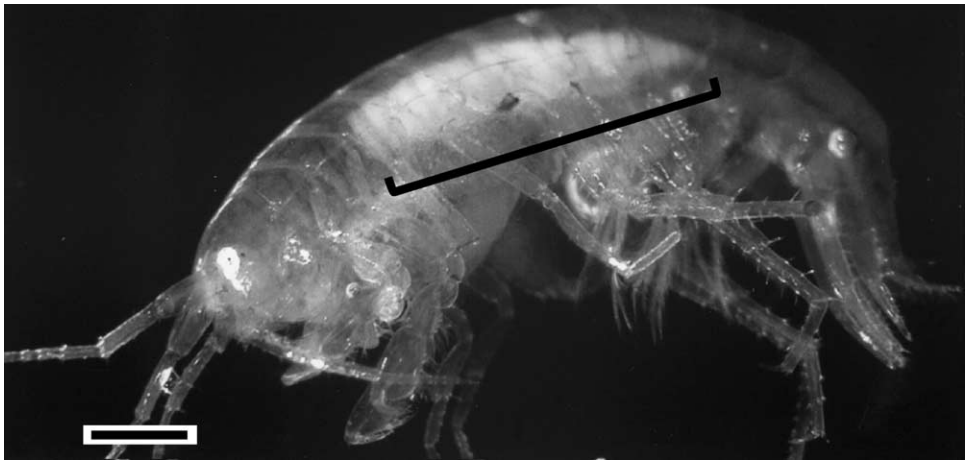

Fig. 1. Adult female *Crangonyx pseudogracilis* with patent *Fibrillanosema crangonycis* infection of the ovaries (bar = 1 mm).

was derived from the parasite or the host. These membranes may have been the product of an immune response by the host after the host cell had been lysed.

Meronts were found in follicle cells and oocytes, never outside the gonad (Figs. 4 and 5). Meronts were amorphous and ranged from approximately 1.5–3.5 μm ($N = 15$) in diameter. The cytoplasm of the diplokaryotic meronts was filled with endoplasmic reticulum and ribosomes. All meronts were in direct contact with the host cell cytoplasm and there was no evidence of grouping or enclosure within a membrane. Meronts were not seen undergoing replication, however, the absence of large merogonial plasmodia suggests that division was likely to be by binary fission.

During early sporogony, the plasma membrane became more electron dense by means of a granular accumulation of protein on the surface (Fig. 7). The early sporoblast displayed expansion of the endoplasmic reticulum and initiation of polar filament formation (Fig. 8). The polar filament and exospore wall were the earliest of the recognisable spore features to differentiate (Fig. 9). Before the endospore and exospore were fully laid down, the sporoblast was sometimes irregularly shaped (Fig. 4). On the surface of the developing sporoblast we observed pockets that contained a fibrillar matrix (Figs. 9 and 10). These may represent localised build up of spore coat surface proteins that will spread to coat the entire spore. By late sporoblast development, the organisation of the spore organelles was evident (Fig. 11).

Mature spores were diplokaryotic and ovoid, measuring 5.61 ± 0.13 μm in length and 2.05 ± 0.08 μm in width ($N = 27$). The cytoplasm contained many rows of ribosomes (Fig. 12) and the extrusion apparatus consisting of the polaroplast, anchoring disc (Fig. 13) and polar filament (Fig. 12). The spore wall consisted of a thick electron lucent endospore wall and a thin electron dense exospore wall that was covered by a proteinaceous coat (Figs. 12 and 13). The anchoring disc lay at the anterior tip of the spore (Fig. 13), where the endospore wall was at its thinnest, and was connected to the manubroid polar filament. The isofilar polar filament passed through the uniformly lamellate polaroplast (Fig. 13) and lay in a single layer, completing 19–26 turns at the outer edge of the spore ($N = 30$, Fig. 12). The posterior vacuole occupied 20% of the posterior portion of the spore and was granular in nature (Fig. 12).

The spores of the novel microsporidium are morphologically similar to spores observed from North American populations of *Crangonyx* (near) *pseudogracilis* (Fig.14) from North Carolina. The diplokaryotic spores, 3.73 × 1.62 μm (LAO, $N = 10$) were restricted to the hepatopancreas with high spore burdens and associated pathology. Additionally, populations of *Crangonyx floridanus* (Fig. 15) from Louisiana were infected with a parasite that was also located in the ovary and resulted in heavy infection. These spores (Fig. 15) were morphologically identical to the parasites found in the UK *Crangonyx* population (Fig. 12) with diplokaryon nucleus, fibrous proteinaceous coat, localised thinning of the endospore wall at the anchoring disc, laminate polaroplast, granular

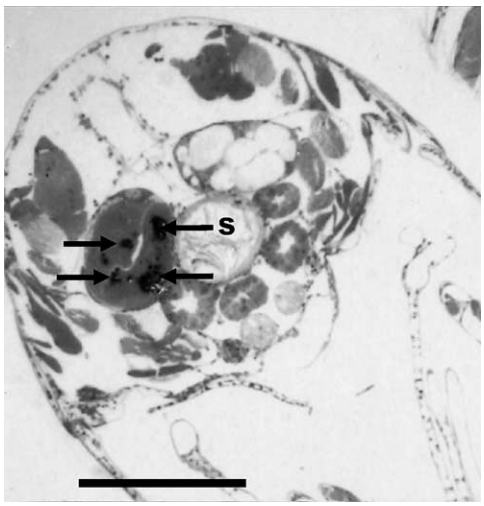

Fig. 2. Light microscopic cross section of *Crangonyx pseudogracilis* with patent infection of microsporidian spores (s) in one ovary (bar = 1 mm).

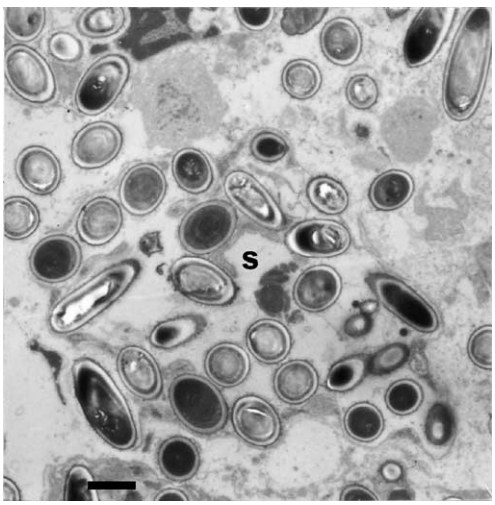

Fig. 3. Electron microscopic cross section showing masses of spores (s) within disrupted ovarian tissue (bar = 2 μm).

posterior vacuole, and similarities in the number and angle of polar filament coils. The spores found in Louisiana populations were larger than those found in the UK with a mean size of 7.08 × 2.68 μm (LAO stain, $N = 10$) in the specimen shown (Fig. 12).

Using the primers 530f::580LSUr, 1400 bp of partial SSU, ITS, partial LSU rDNA were amplified. Parasites were found primarily in female gonads and in embryos but were less frequent in males and in the tissue surrounding the gonads (Table 3). These primers did not amplify any other

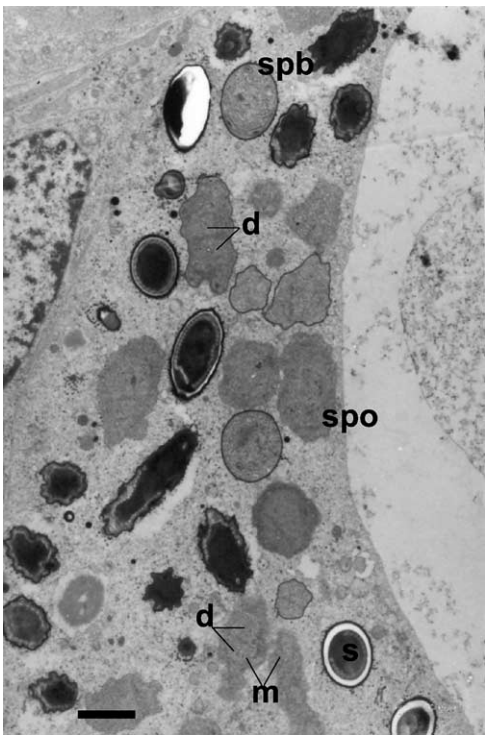

Fig. 4. Follicle cell containing diplokaryotic (d) meronts (m), sporonts (spo), sporoblasts (spb) and spores (s) (bar = 2 μm).

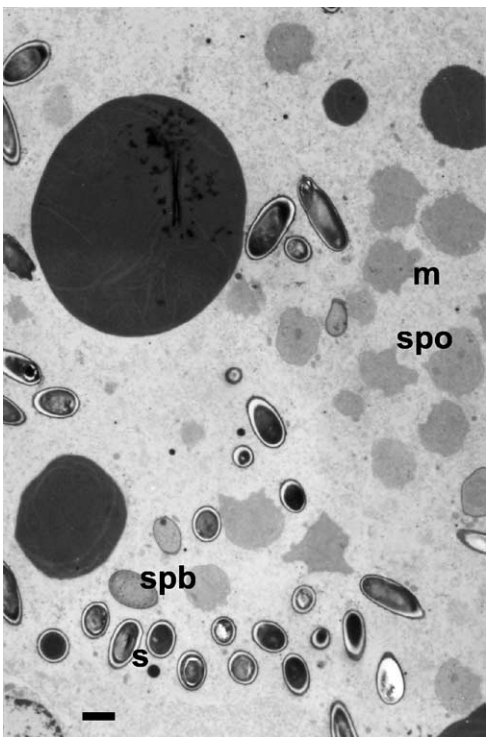

Fig. 5. Oocyte containing diplokaryotic meronts, sporonts, sporoblasts and spores (bar = 2 μm).

microsporidia from this host. No amplification was obtained from negative controls.

A FASTA search revealed that the novel microsporidium was most closely related to a sequence submitted as *Visvesvaria acridophagus* (AF024658), *Thelohania solenospae* (AF134205), and *Brachiola algerae* (= *N. algerae* AF069063) on GenBank. Sequence alignment with BioEdit (Hall, 2001) revealed 88, 77 and 77% sequence similarity, respectively. Phylogenetic analyses using parsimony, neighbour joining, and maximum likelihood criteria

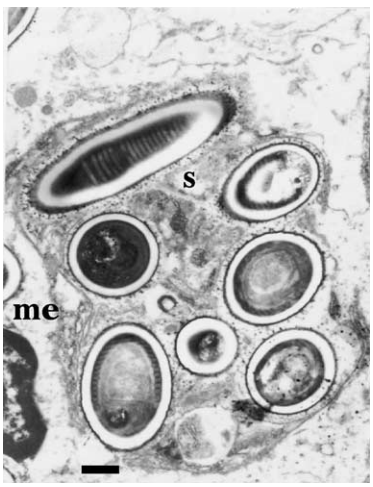

Fig. 6. Group of *Crangonyx pseudogracilis* spores (s) in infected ovary surrounded by membrane (me) (bar = 1 μm).

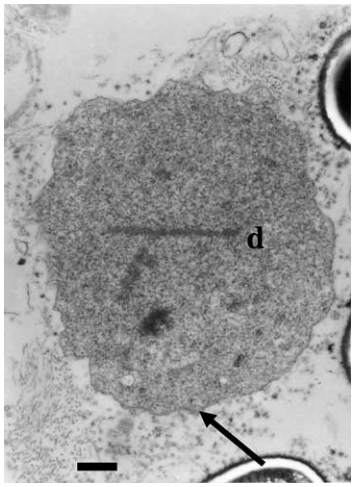

Fig. 7. Early sporont exhibiting diplokaryotic nucleus (d) and initiation of membrane thickening (arrow) (bar = 500 nm).

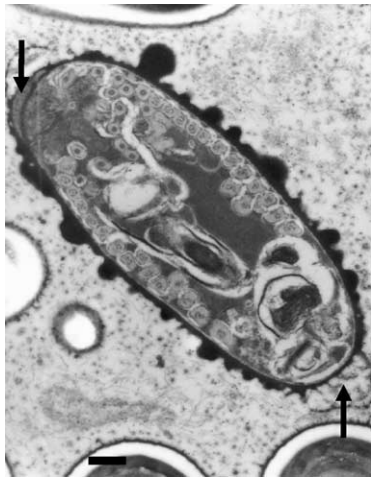

Fig. 9. Developing sporoblast showing deposition of fibrillar matrix (arrows) (bar = 500 nm).

all resulted in identical trees that placed our novel sequence outside extant genera in an uncharted area of the tree (Fig. 16). Only the maximum likelihood tree has been presented (Fig. 16).

4. Discussion

The morphological and molecular data support the creation of a new genus for this novel microsporidian parasite. We therefore erect the genus *Fibrillanosema*, taking the name from the fibrillar matrix, involved in spore wall formation together with ultrastructural homology of observed life cycle stages to members of the genus *Nosema*. These characteristics include a diplokaryon nucleus, oval spores, and disporoblastic development with all lifecycle stages in direct contact with the host cytoplasm. However our molecular data places *F. crangonycis* alongside *B. algerae* and *T. solenopsae* and the recently erected family Pseudonosematidae which includes *Pseudonosema cristatellae* and *Bryonosema plumatellae* parasites of Bryozoa. These parasites exhibit morphological and life-cycle features characteristic of the Nosematidae demonstrating that this phenotype is represented in more than one lineage. The sequence divergence supports placement of *Fibrillanosema* in a new genus however the paucity of data in this area of the phylogenetic tree make it impossible to assign higher-level taxa. Further support for the new genus comes from a morphological trait, the unusual pockets of fibrillar material on the surface of the developing sporoblasts.

An unusual feature of the microsporidian infection is the high level of pathogenesis caused by a parasite that is restricted to the ovaries and has been shown to utilise vertical transmission. A parasite that is vertically transmitted depends on host reproduction for its transmission, hence high pathogenesis and associated reduction in host

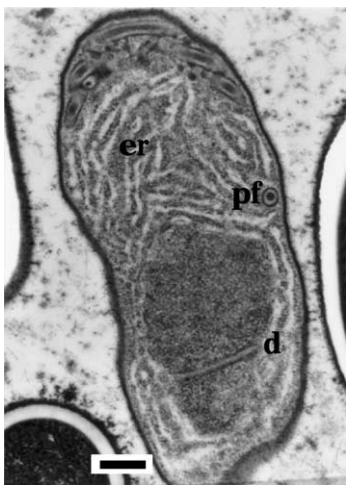

Fig. 8. Sporoblast exhibiting diplokaryotic nucleus (d) abundant endoplasmic reticulum (er) (bar = 500 nm).

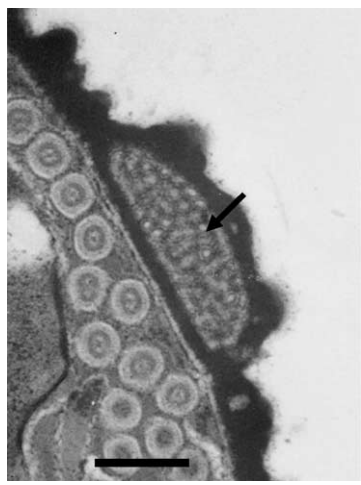

Fig. 10. Deposition of fibrillar matrix (arrow) (bar = 250 nm).

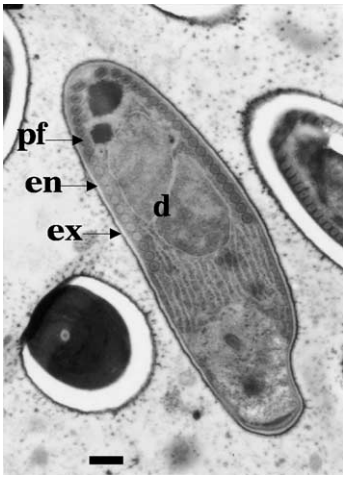

Fig. 11. Late sporoblast with diplokaryotic nucleus (d) mature polar filament (pf) and developing endospore (en) and exospore (ex) walls (bar = 1 μm).

reproductive fitness should be selected against (Dunn and Smith, 2001). Indeed other vertically transmitted microsporidia found in European amphipods are characterised by low burden and low pathogenesis (Terry et al., 1999; Ironside et al., 2003). The high parasite burden and absence of the FC/autoinfection spore previously associated with vertical transmission (Iwano and Ishihara, 1991; Ni et al., 1995; Johnson et al., 1997; Terry et al., 1999) imply that *F. crangonycis* employs horizontal transmission in addition to the demonstrated vertical route (Mangin et al., 1995). In microsporidia that utilise mixed transmission strategies, there is evidence of higher levels of virulence during the transovarial phase of the life cycle than in those that use strictly vertical transmission (Dunn and Smith, 2001). The pathology in the ovary is likely to lead to reduced host fertility or even castration and associated loss of vertical transmission of the parasites. The microsporidium may utilise vertical transmission as long as the ovaries are functional and switch to horizontal transmission upon host death.

The invasive species *C. pseudogracilis* occurs in freshwater habitats with the native gammarids *G. pulex*, *Gammarus lacustris*, and *G. duebeni celticus* (Gledhill et al., 1993). *Fibrillanosema crangonycis* is morphologically distinct from all previously described microsporidia from these native amphipods (Table 1) so it is unlikely that *C. pseudogracilis* has become infected with *F. crangonycis* through transmission from sympatric hosts. However, there are several uncharacterised microsporidia from the host genus *Crangonyx* in North America (Figs. 14 and 15) that show close structural homology to *F. crangonycis*. The similarity in structure leads us to conclude that this microsporidium has been introduced with its host from North America. While *F. crangonycis* is morphologically distinct and displays different infection patterns to microsporidia from native European populations, we must consider the possibility that the microsporidium may be transmittable to these native amphipods. The possibility of transmission to native hosts is increased by their predation on the invasive *C. pseudogracilis* (MacNeil et al., 2003a). This could be of serious consequence to native amphipods as microsporidia that are able to infect multiple host species may not necessarily utilise the same transmission strategies. Thus, while *F. crangonycis* is vertically transmitted in its original host, it could adopt a completely different infection strategy when introduced into novel hosts.

In light of recent work suggesting that invaders lose their parasites (Torchin et al., 2002, 2003), it is perhaps surprising that this microsporidian infection has not only persisted following invasion into a novel habitat by the host, but also that prevalence is very high. There are several reasons why parasites may be lost during an invasion event. Embryonic or larval invaders are rarely parasitised (Lafferty and Kuris, 1996) or low founder numbers and population bottlenecks (Dove and Ernst, 1998) may limit the spectrum of parasites (Torchin et al., 2003). Additionally, the absence of alternative hosts in complex life cycles (Cribb et al., 2000) or adverse environmental conditions may limit parasite establishment (Kelly et al., 2002). While horizontally transmitted parasites are vulnerable to these mechanisms of enemy release, the implications for vertically transmitted parasites have not been considered. Vertical transmission is an effective mechanism of dispersal over long distances or in patchy habitats (Dunn et al., 2001). Additionally, the reduced virulence associated with vertical transmission may enhance parasite survival and maintenance within host founder populations (Dunn et al., 1995; Dunn and Smith, 2001). Therefore, it is of particular interest that *F. crangonycis* is vertically transmitted between generations of its *C. pseudogracilis* host.

To increase their transmission success, vertically transmitted parasites are frequently associated with host sex ratio distortion (Hatcher and Dunn, 1995; Terry et al., 1998; Bandi et al., 2001; Ironside et al., 2003). Our data show significant over representation of *F. crangonycis* in females, which is supportive of sex ratio distortion by the parasite. The likelihood of success during population establishment of an invasive host may be increased by parasitic sex ratio distortion as overproduction of females causes a higher rate of population increase (Hatcher et al., 1999). Thus, we predict that invaders with vertically transmitted parasites are less likely to escape their ‘enemies’ and that those harbouring feminising parasites may benefit from increased invasion success.

5. Taxonomic summary

5.1. Fibrillanosema n.g

Nuclei are in diplokaryotic arrangement throughout the life cycle. Parasite stages are in direct contact with the host

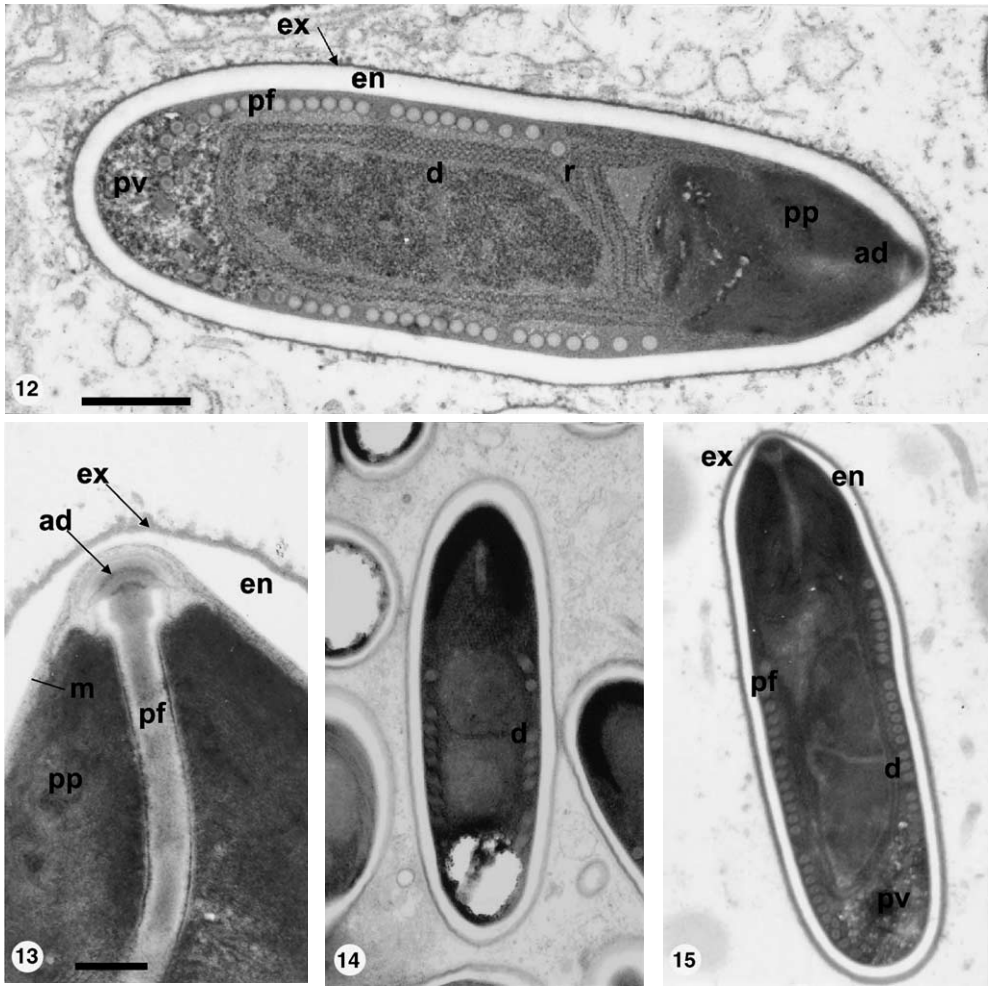

Plate 1

Fig. 12. *Crangonyx pseudogracilis* spore with diplokaryotic nucleus (d) isofilar polar filament (pf), polaroplast (pp), polyribosomes (r) posterior vacuole (pv) polar filament (pf) and mature endospore (en) and exospore (ex) walls (bar = 1 μm).

Fig. 13. Detail of *Crangonyx pseudogracilis* spore showing polar filament (pf) with anchoring disc (ad), polaroplast (pp) and parasite membrane (m) with endospore (en) and exospore (ex) walls (bar = 200 nm).

Fig. 14. Diplokaryotic microsporidian spore in *Crangonyx* (near) *pseudogracilis* from a population in North Carolina showing diplokaryon nucleus (d).

Fig. 15. Diplokaryotic microsporidian spore in the ovary of *Crangonyx floridanus* from a population in Louisiana showing diplokaryon nucleus (d), polar filament (pf), posterior vacuole (pv), exospore (ex) and endospore (en).

cytoplasm. Merogonial divisions are probably by simple fission of elongated cells. No plasmodial stages were observed. Division during sporogony was not observed. Endospore wall was thinner over anchoring disc. The diplokaryon was surrounded by numerous rows of isofilar polar filament coils arranged in a single row. Generic name based on *Nosema* with the prefix *Fibrilla* meaning 'small fibre'.

5.1.1. Fibrillanosema crangonycis

- *Type host*. The freshwater amphipod *C. pseudogracilis*.
- *Transmission*. Transovarial; cannot exclude horizontal.
- *Site of infection*. Gonadal tissue of adult hosts.
- *Interface*. All stages of the parasite are found in direct contact with the host cytoplasm. Infrequently, a membrane of unknown origin surrounds spores in follicle cells.
- *Other host-parasite cell relations*. Parasites are found diffuse within the ovary with no obvious xenoma formation.
- *Merogony*. Diplokaryotic meronts. Division is likely to be by binary fission.
- *Transition to sporogony*. Granular material appears on the thickening plasma membrane.
- *Sporogony*. The sporont is an ovoid or irregularly shaped diplokaryotic cell.
- *Spore*. The binucleate, ovoid spore measures 5.61 ± 0.13 μm in length and 2.05 ± 0.08 μm in width when fixed. The exospore wall is thin but covered in a proteinaceous coat and the endospore wall is thick. The polaroplast is lamellate in structure, the isofilar polar filament has up to 26 turns, and the granular posterior vacuole takes up to 20% of the cell.

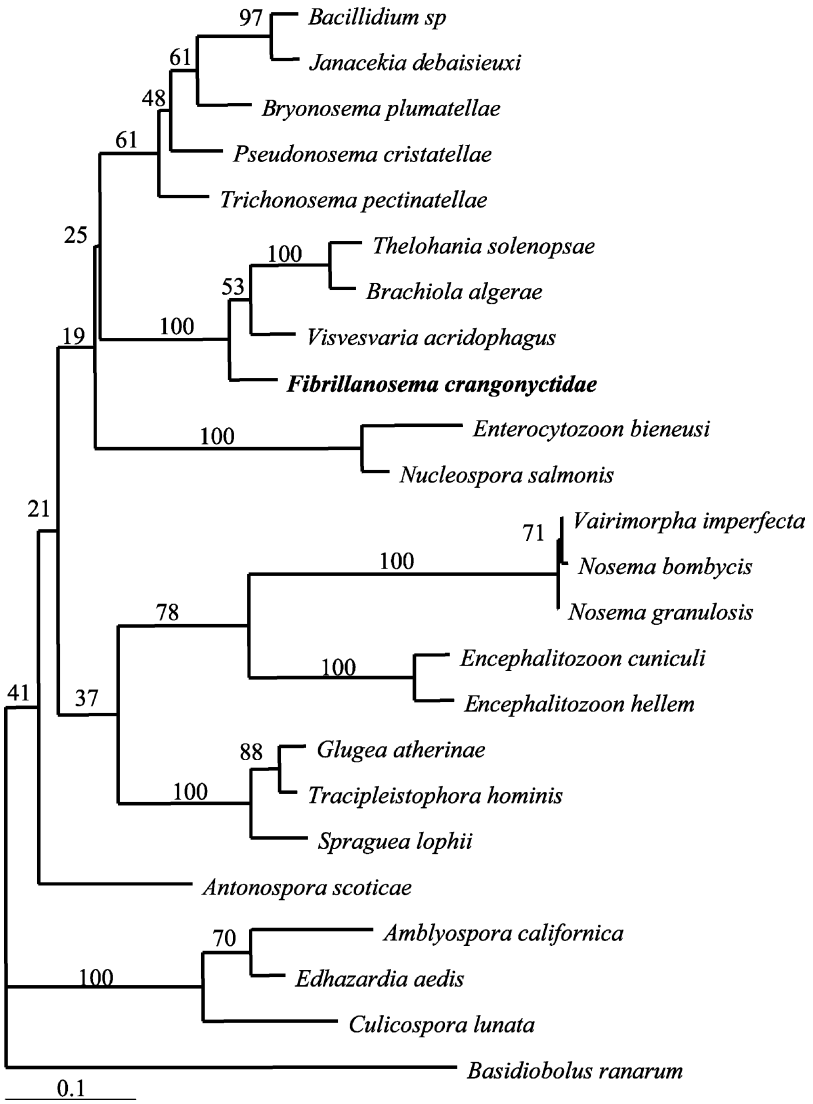

Fig. 16. Phylogenetic tree of microsporidia based on partial SSU, ITS, and LSU rDNA using maximum likelihood, showing the position of *Fibrillanosema crangonycis*. Bootstrap analysis (100 replicates) of the most parsimonious tree. The bootstrap values on the tree represent the percentage of bootstrap replicates that gave that topology. Neighbour joining and maximum parsimony result in identical phylogenetic trees.

- *Type locality*. Middleton Park, Leeds, West Yorkshire, UK (1° 32.5′ W 53° 45.1′N).
- *Remarks*. A key feature of the parasite is the presence of pockets of fibrillar material on the surface of the developing sporoblasts. The microsporidium is highly pathogenic despite being found almost solely in the ovaries of adult female hosts. A holotype slide, consisting of a semi-thin section of infected female *C. pseudogracilis* gonad stained with toluidene blue, has been placed in the Natural History Museum, Cromwell Rd, London SW7 5BD, Accession No. 2003.10.22.1. The nucleotide sequence of *F. crangonycis* has been deposited in the GenBank database under Accession No. AY364089. *Fibrillanosema crangonycis* n.sp., refers to the type host, *C. pseudogracilis*, from which it has been described.

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